COMPOSITION AND METHOD TO PREVENT OR REDUCE DIARRHEA AND STEATORRHEA IN HIV PATIENTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending application Serial No. 10/100,716 filed on March 17, 2002.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a composition and method using the composition to treat and to prevent/reduce diarrhea and steatorrhea in HIV patients who are treated with High Activity Antiretroviral Therapy, hereinafter referred to as "HAART".

Reported Developments

The current most effective treatment of individuals infected with Human Immunodeficiency Virus, hereinafter referred to as "HIV", is the HAART method which comprises administering a combination of drugs that attack the HIV mechanism for viral reproduction. The therapy consists of using drugs that inhibit reverse transcriptase and HIV protease. HAART is intended to increase CD4 lymphocyte counts and suppression of HIV load in response to the antiretroviral therapy. Ultimately, the therapy results in declining HIV-related morbidity and mortality.

Drugs used in HAART include: protease inhibitors (PI); non-nucleoside reverse transcriptase inhibitors (NNRTI); and nucleoside reverse transcriptase inhibitors (NRTI). Table I lists these drugs by trade name, chemical name and type.

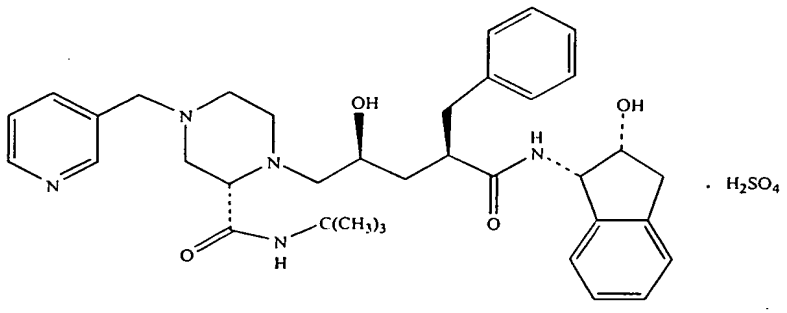
TABLE I

DRUGS USED IN HAART

Trade Name	Chemical Name	Type
CRIXIVAN®	Indinavir sulfate	PI
AGENERASE®	Amprenavir	PI
NORVIR®	Ritonavir	PI
FORTOVASE®	Saquinavir	PI
VIRACEPT®	Nelfinavir mesylate	PI
INVIRASE®	Saquinavir mesylate	PI
SUSTIVA®	Efavirenz	NNRTI
VIRAMUNE®	Nevirapine	NNRTI
ZIAGEN®	Abacavir sulfate	NNRTI
RESCRIPTA®	Delavirdine mesylate	NNRTI
HIVID®	Zalcitabine	NRTI
ZERIT®	Stavudine	NRTI
RETROVIR®	Zidovudine	NRTI
EPIVIR®	Lamivudine	NRTI
COMBIVIR®	Lamivudine, Zidovudine	NRTI
VIDEX®	Didanosine	NRTI

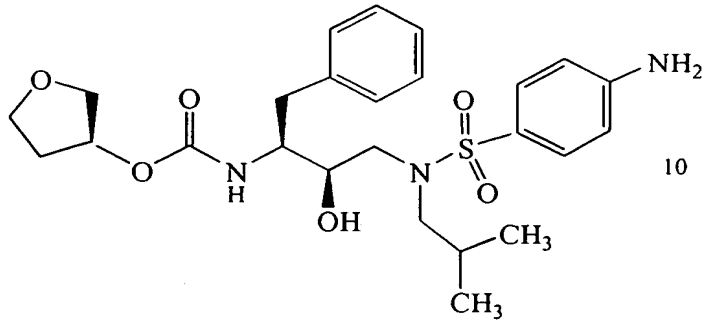
The chemical names and formulas are described hereunder.

Indinavir Sulfate. 2,3,5-Trideoxy-*N*-[(1S,2R)-2,3-dihydro-2-hydroxy-1*H*-inden-1-yl]-5-[(2S)-2-[[1,1-dimethylethyl)amino]carbonyl]-4-(3-pyridinylmethyl)sulfate, having the formula:



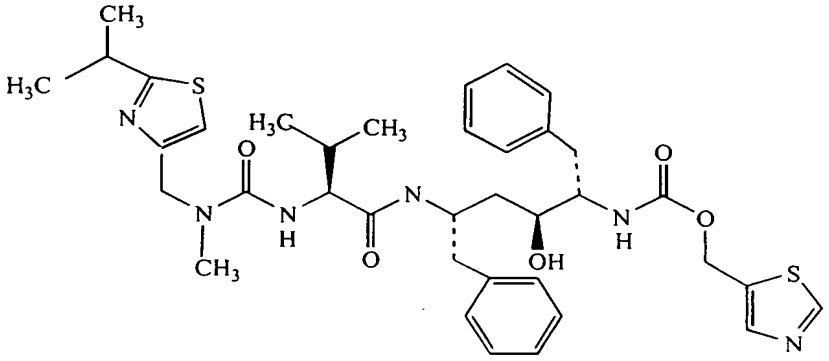
The preparation of the compound is described in U.S. Patent No. 5,413,999.

Amprenavir. [(1S,2R)-3-[[4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]carbamic acid (3S)-tetrahydro-3-furanyl ester, having the formula:



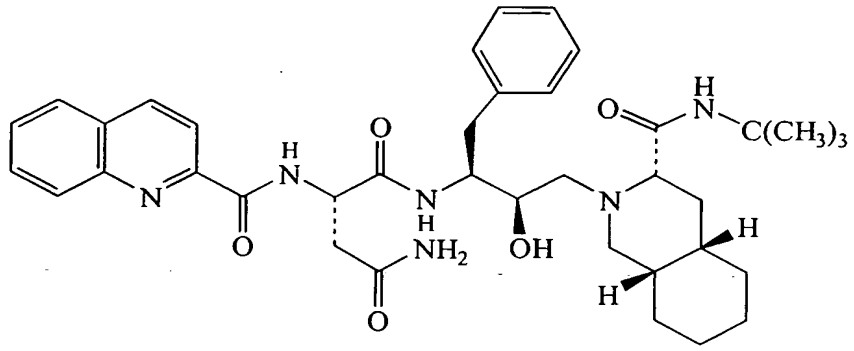
The preparation of the compound is described in U.S. Patent No. 5,585,397.

Ritonavir. 10-Hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid 5-thiazolylmethyl ester, having the formula:



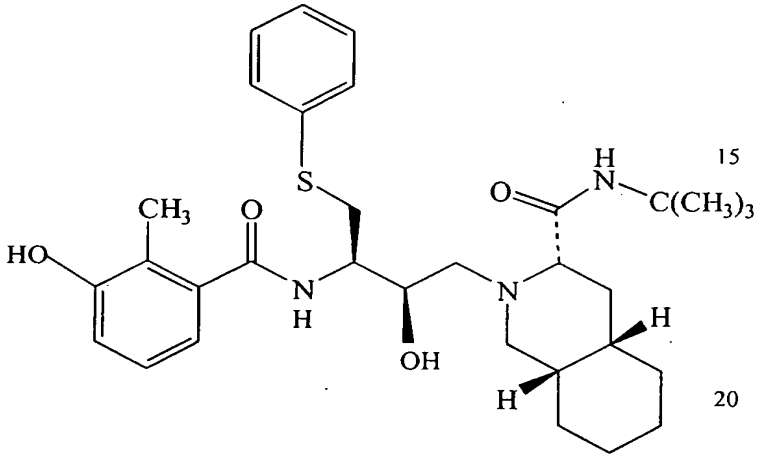
The preparation of the compound is described in U.S. Patent No. 5,541,206.

Saquinavir. N-*tert*-butyldecahydro-2-[2(R)-hydroxy-4-phenyl-3(*S*)-[[N-(2-quinolycarbonyl)-L-asparaginyl]-amino]butyl](4aS,8aS)-isoquinoline-3(S)-carboxamide, having the formula:



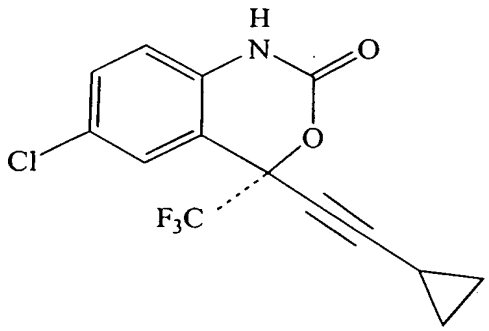
The preparation of the compound is described in U.S. Patent No. 5,196,438.

Nelfinavir. 2-[2'-hydroxy-3'-phenylthiomethyl-4'-aza-5'-oxo-5'-(2"-methyl-3"-hydroxyphenyl)pentyl]decahydroisoquinoline-3-*N*-*t*-butyl-carboxamide, having the formula:



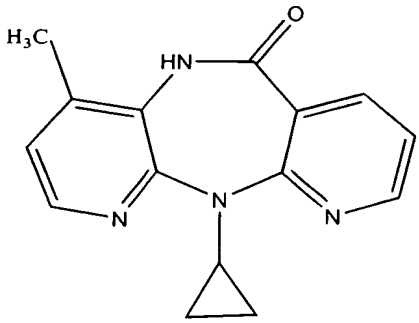
The preparation of the compound is described in U.S. Patent No. 5,484,926

Efavirenz. 6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benoxazin-2-one, having the formula:



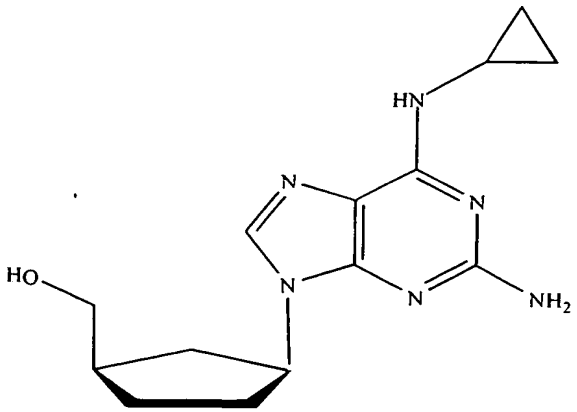
The preparation of the compound is described in U.S. Patent No. 5,519,021.

Nevirapine. 11-Cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido[3,2-b:2',3'-e][1,4]diazepin-6-one, having the formula:



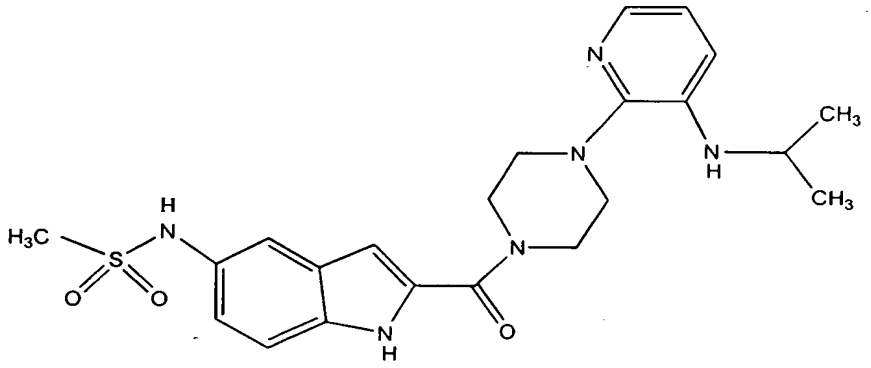
The preparation of the compound is described in U.S. Patent No. 5,366,972.

Abacavir. 4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2-cyclopentene-1-methanol, having the formula:



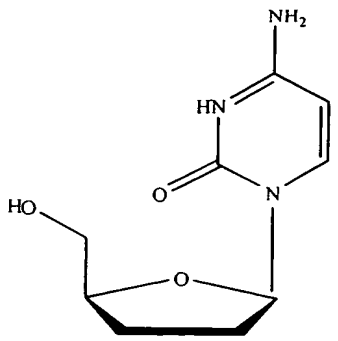
The preparation of the compound is described in U.S. Patent No. 5,034,394. The sulfate form of the compound is ZIAGEN®.

Delavirdine. 1-[3-[(1-Methylethyl)amino]-2-pyridinyl]-4-[[5-[(methylsulfonyl)amino]-1H-indol-2-yl]carbonyl]piperazine, having the formula:



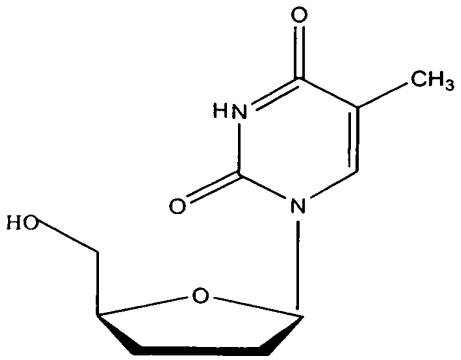
The preparation of the compound is described in WO 91/09849.

Zalcitabine. 2',3'-Dideoxycytidine, having the formula:



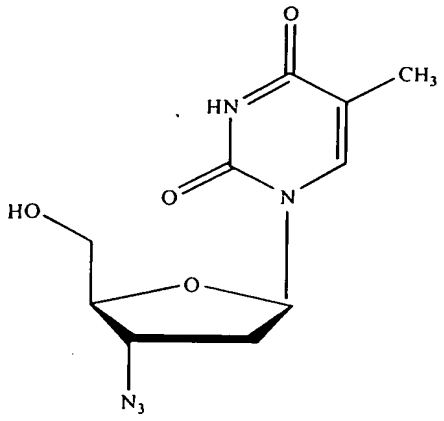
The preparation of the compound is described in J.P.Horwitz et al., *J. Org. Chem.*, 32, 817 (1967).

Stavudine. 2',3'-Didehydro-3'-deoxythymidine, having the formula:



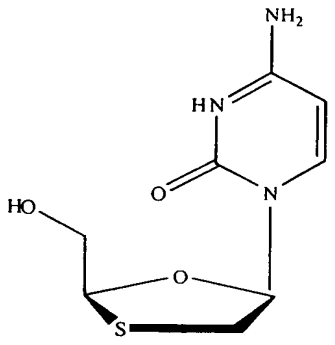
The preparation of the compound is described in U.S. Patent No. 5,130,421.

Zidovudine. 3'-Azido-3'-deoxythymidine, having the formula:



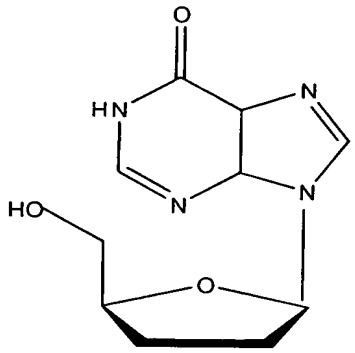
The preparation of the compound is described in U.S. Patent No. 4,724,232.

Lamivudine. (2R-cis)-4-Amino-1-[2-hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone, having the formula:



The preparation of the compound is described in WO 91/17159.

Didanosine. 2',3'-Dideoxyinosine, having the formula:



The preparation of the compound is described in EP206497.

Using HAART, a combination of these drugs, often referred to as "cocktails", is administered to HIV patients. The Panel on Clinical Practices convened by the Department of Health and Human Services (DHHS) and the Henry J. Kaiser Family Foundation has recently developed guidelines for the treatment of HIV. Table II shows the recommended antiretroviral therapy for initial treatment of HIV patients.

TABLE II

RECOMMENDED ANTIRETROVIRAL THERAPY FOR INITIAL TREATMENT OF HIV PATIENTS

Recommendation	PI or NNRTI	NRTI
Strongly recommended	Efavirenz (NNRT) Indinavir (PI) Nelfinavir (PI) Ritonavir + Saquinavir (PI)	Stavudine + Lamivudine Stavudine + Didanosine Zidovudine + Lamivudine Zidovudine + Didanosine
Recommended as Alternative	Abacavir (NNRTI) Amprenavir (PI) Delavirdine (NNRTI) Nelfinavir + Saquinavir (PI) Nevirapine/Ritonavir (PI) Saquinavir (PI)	Didanosine + Lamivudine Zidovudine + Zalcitabine

Many individuals infected with HIV and receiving HAART suffer from mild to severe diarrhea which is a side effect of the treatment. A recent study (Kakuda et al., "Protease Inhibitors for the treatment of human immunodeficiency virus infection", *Am. J. Health Syst. Pharm.*, vol. 55, no. 3, pp. 233-254, February 1, 1998) states that 12-20% of HIV patients receiving nelfinavir experience diarrhea. It has also been reported that 75% of HIV-1 positive individuals that had not previously received antiretroviral therapy had episodes of diarrhea after starting a quadruple regimen of stavudine and lamivudine with nelfinavir and saquinavir (Reijers, MH et al., "Toxicity and drug exposure in a quadruple drug regimen in HIV-1 infected patients participating in the ADAM study", *AIDS*, vol. 14, no. 1, pp. 59-67, January 7, 2000). The detrimental effects of diarrhea include maldigestion, malabsorption of nutrients, excretion of undigested fats (steatorrhea), and unabsorbed pharmaceuticals resulting in decreased immunocompetance, and loss of muscle mass (Sherman, DS, et al., "Management of protease inhibitor-associated diarrhea", *Clin, Infect. Dis.*, vol., 30, no. 6, pp. 908-914, 2000).

Drug-induced diarrhea diminishes the overall therapeutic effectiveness of the HAART drugs by hindering their absorption into the patient's circulatory system. In addition, the overall quality of life of the patient is severely compromised (Watson, A., "Diarrhea and quality of life in ambulatory HIV-infected patients", *Dig. Dis. Sci.*, vol. 41, no. 9, pp. 1794-1800, September 1996). Due to the reduction in quality of life, compliance with drug therapy is often a serious problem. Current treatments for diarrhea of HIV-positive patients on HAART therapy include oat bran, psyllium, loperamide, calcium carbonate and other over-the-counter medications that are only partially effective. There is insufficient information on the mechanism of antiretroviral drug-induced diarrhea and steatorrhea. It is hypothesized that the combination of drugs in HAART may interfere with the production and release of the digestive components by directly inhibiting the enzyme activating cascade and/or the digestive enzymes, both lipase and proteases, or disrupt complexation of lipase with colipase or bile salts.

More than 95% of dietary fats ingested by the average adult in a day are triglycerides, and if these nutrients remain undigested, diarrhea can result. The digestion of triglycerides is chemically complicated and involves two fundamentally different but closely interrelated processes: the activation of several inactive proenzymes (zymogens), and the emulsification of nutrient lipids with bile salts. In the first part of the process, trypsin converts the zymogen

procolipase, secreted by the pancreas, into the 12 kD protein colipase. In the second part of the process, colipase anchors the complex formed between a lipase and a micellar bile acid to its triglyceride substrate, thereby stabilizing the complex and activating it enzymatically; the triglyceride may now be hydrolyzed to free fatty acids and monoacyl glycerol. The trypsin needed in the first part of the process is produced by the pancreas as trypsinogen (zymogen) and activated by enterokinase, which is secreted by the Brunner's gland in the duodenum. The inhibition of pancreatic lipase or any enzyme in the zymogen-activating cascade leads to undigested fats that become hydroxylated to hydroxy fatty acids by the intestinal bacterial flora. Hydroxylated fatty acids are well-known diuretics.

SUMMARY OF THE INVENTION

It has now been discovered that exogenous administration of a bicarbonate-buffered and enteric-coated pancrelipase to human immunodeficiency virus (HIV) positive patients who experience diarrhea due to HAART drugs which contain nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI), or non-nucleoside reverse transcriptase inhibitors (NNRTI) reduces/eliminates diarrhea and/or steatorrhea.

The bicarbonate-buffered and enteric-coated pancrelipase maybe co-administered or sequentially administered with protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), or non-nucleoside reverse transcriptase inhibitors (NNRTI) to patients who are HIV-positive.

The protease inhibitors (PI), nucleoside reverse transcriptase inhibitors (NRTI), and non-nucleoside reverse transcriptase inhibitors (NNRTI) include the following drugs listed by their chemical names: Indinavir sulfate, Amprenavir, Ritonavir, Saquinavir, Nelfinavir mesylate, Saquinavir mesylate, Elfavirenz, Nevirapine, Abacavir sulfate, Delavirdine mesylate, Zalcitabine, Stavudine, Zidovudine, Lamivudine, Lamivudine/Zidovudine combo and Didanosine.

The bicarbonate-buffered and enteric-coated pancrelipase (disclosed in U.S. Patent No. 5,578,304 and incorporated herein by reference in its entirety) preferably comprises:

(a) from about 10 to about 90% w/w of an enzyme selected from the group consisting of pancreatic proteases, lipases, co-lipase, nucleases, amylases, and bio-active substances produced by the pancreatic gland;

(b) from about 15 to 60% w/w of a buffering agent selected from the group consisting of anhydrous sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, ammonium carbonate, tromethamine, di(tris)hydroxymethylaminomethane-carbonate, tris-glycine, di-arginine, tri-arginine, poly-arginine, di-lysine, tri-lysine, poly-lysine, diethylamine and triethanolamine, said buffering agent providing a pH of from 7 to 9 in the small intestine of a mammal, and said lipase having an activity of from 24% to 100% at said pH of from 7 to 9;

(c) from about 0.5 to about 16% w/w of a disintegrant selected from the group consisting of ursodiol, starch, modified starches, microcrystalline cellulose and propylene glycol alginate;

(d) from about 1 to about 19% w/w of an adhesive polymer selected from the group consisting of polyvinylpyrrolidone, hydroxyethyl cellulose, cellulose acetate phthalate, ethyl cellulose and hydroxypropylmethyl cellulose;

(e) from about 7.0 to about 15 % w/w of a non-porous, gastric acid-resistant and pharmaceutically acceptable polymer coating which contains less than 2% talc and which is insoluble in the pH range of from about 1.5 to about 5 but is soluble in the pH range of about 5.5 to about 9, said polymer coating comprises a polymer selected from the group consisting of hydroxypropyl methyl cellulose phthalate, cellulose acetate phthalate, diethyl phthalate, dibutyl phthalate, and enteric coating polymer dispersion, and an acrylic based polymeric dispersion.

In the method of treatment of the present invention, the term "subject" refers to a human who has been the subject of treatment, observation or experiment.

The "therapeutically effective amount" is that amount of the combination of agents taken together so that the combined effect elicits the desired biological or medical response.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows stool frequency/24 hours of patients before and during PANCRECARB® therapy;

FIG. 2 shows that 87% of HIV patients had a reduced number of stools/24 hours while taking PANCRECARB®;

FIG. 3 shows rate of interference with work of patients before and while taking PANCRECARB®;

FIG. 4 shows that 75% of HIV-positive subjects experienced a decrease in the rate of interference with work while taking PANCRECARB®;

FIG. 5 shows urgency of bowel movement before/during PANCRECARB® therapy;

FIG. 6 shows rate of urgency of bowel movement before PANCRECARB® therapy;

FIG. 7 shows rate of urgency of bowel movement during PANCRECARB® therapy;

FIG. 8 shows stool consistency during PANCRECARB® therapy; and

FIG. 9 shows quality of life during PANCRECARB® therapy.

DETAILED DESCRIPTION OF THE INVENTION

Methods Employed – In Vitro Studies

Enzyme and Drug Preparation of Lipase, Protease and Trypsin Assays

The enzyme sources consisted of USP Reference Standard Pancrelipase, USP Reference Standard Trypsin, colipase (Sigma Chemical Co.), and enterokinase (ICN Biochemicals). Appropriate concentrations of enzyme reference standard were prepared in cold distilled water, as described in USP reference assay procedures. The Infectious Disease Clinic in Somerville, NJ provided the HAART medications. Pure drugs could not be obtained so commercial drug preparations were used in the assays. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was provided by Eastman Chemical Co. (Anglesey, UK); Vitamin E (HealthSmart Vitamins) was purchased from Eckerd Drugs (Bethlehem, PA); povidone was provided by ISP Technologies, Calvert City, KY. Appropriate concentrations of HAART drug, drug cocktails, and excipients were prepared in distilled water and reagent-grade methanol (Aldrich, Milwaukee, WI) or DMSO (Aldrich, Milwaukee, WI).

Lipase Assay

One unit (U) of lipase activity is defined as the amount of enzyme that liberates one μequivalent of free fatty acid from triglycerides per minute at pH 9.0 and 37°C. Lipase activity (U/mg) was determined by a lipase specific titrimetric assay described in the United States Pharmacopeia (USP), Volume XXII. In brief, the substrate consists of 10% olive oil in acacia solution, 40-mg/mL sodium taurocholate, 0.075M calcium chloride, 0.05M Tris in 3.0M NaCl buffer solution (pH 7.5) and distilled water. The emulsified substrate is incubated at 37°C and brought to pH 9.0 with 0.02N NaOH. At time zero, 1.0 mL of enzyme solution was added. A pH stat autotitrator was used to maintain pH 9.0 and the volume of 0.02 N NaOH dispensed per unit time was recorded for five (5) minutes. From the initial slope of this plot, lipase activity was calculated using the following equation:

$$U/mg = \frac{\text{slope (mL of NaOH dispensed/min)} \times \text{N of NaOH} \times 1000}{\text{mg of enzyme assayed}}$$

HAART drug solutions were mixed with the substrate prior to incubation and addition of enzyme. Orlistat, a known potent inhibitor of lipase was used as a positive reference control.

Recovery of Pancrelipase Activity after Inhibition by Protease Inhibitors

The above procedure was used to monitor pancrelipase activity with the exception that drug was not preincubated with the substrate. At time zero 1.0 mL of pancrelipase was added to initiate the reaction. At five (5) minutes, 1.0 mL of HAART drug solution was added to the reaction mixture to cause inhibition of lipase activity. At twelve (12) minutes an additional 1.0 mL of pancrelipase was added to the reaction mixture to overcome HAART inhibition. The reaction was monitored for twenty (20) minutes.

Pancrelipase Reactivation by Colipase

Junge et al., described the procedure for the reactivation of lipase by colipase. In brief, the above lipase assay procedure was used with the following variation: colipase solution replaced the distilled water so the concentration of colipase in the final reaction mixture was 5 μg/mL.

Trypsin Assay

One unit (U) of trypsin activity is defined as the amount of enzyme causing a change in absorbance of 0.003 per minute from a substrate under the conditions specified in the assay. Trypsin activity (U/mg) was determined by a trypsin specific spectrophotometric assay as described by Bergmeyer. In brief, 0.75 mL triethanolamine (TEA) solution (0.2 M TEA, 0.02M Ca_2Cl, pH 7.8) was mixed with 0.075 mL drug solution (drug in either DMSO or water) and after mixing, 0.025 mL of trypsin solution (0.32 mg/mL in 1.0 mM HCl) was added and this mixture was incubated at 25°C for one minute. At time zero, 0.20 mL benzoyl-L-arginine-4-nitroanilide substrate (0.8 mM in 40% DMSO, 60% TEA solution) was added to give an assay volume of 1.05 mL. This gave final concentrations of substrate, trypsin and drug equal to 2.5 mg/mL, 7.6 μg/mL, and 2.1 * 10-4M respectively. The change in absorbance at 405 nm was monitored over time for five (5) minutes to check for linearity and product inhibition. Each subsequent run was determined over a one-minute span. Trypsin activity was calculated using the following equation:

$$U = \frac{\Delta\text{Absorbance} * \text{Assay volume (1.05 mL)} * 1000}{1.02*10*\text{change in time (1 min)}}$$

HAART drug solutions were mixed with the TEA solution prior to incubation.

Enterokinase Assay

One unit (U) of enterokinase activity is defined as the amount of enzyme that will produce an increase of absorbance of 0.001 per minute at 253 nm from substrate at pH 5.6 and 25°C. The activity of enterokinase was determined by a coupled (enterokinase-trypsin) enzyme assay as provided by Biozyme. In brief, the substrate consisted of trypsinogen (1.2 mg/mL in 1.0 mM HCl/5.0 mM Ca_2Cl). A series of test tubes were labeled for each enzyme preparation and enzyme blank. Each tube contained substrate (trypsinogen) that was equilibrated to 25°C with 0.07 M sodium succinate buffer, pH 5.6 and distilled water. To each reaction tube enterokinase solution (57.6 units/mL buffer) was added and distilled water was added to blank tubes. The total volume contributions of each component were: 1.0 mL succinate buffer, 0.8 ml distilled water, 0.1 mL of enterokinase, 0.5 mL trypsinogen, and 0.2 mL of either; drug dissolved in DMSO, drug dissolved in water, or DMSO/water as control to yield a total reaction volume of 2.6 mL. This gave reaction concentration of tryspinogen, drug, and enterokinase equal to 0.23 mg/mL, 2.2* 10-4M, and 2.0 U/mL respectively. All tubes were incubated for thirty (30) minutes for the enterokinase catalyzed reaction to occur. After incubation, the reaction was quenched with 2.0 M HCl. The test and reaction solutions were added separately to N-benzoyl-L-arginine ethyl ester hydrochloride (0.25 mM in 0.067 M potassium phosphate, pH 7.6) and monitored spectrophotometrically for an increase in absorbance at 253 nm for five (5) minutes to monitor the trypsin catalyzed reaction. Units of enterokinase activity were calculated by using the following equation:

$$U/mL = \frac{\Delta A_{253}/\text{time (min) Test} - \Delta A_{253}/\text{time (min) Blank} \times 5.4}{0.003 \times 0.002 \times (\text{P.A.}) \times 0.024 \times 15 \times 0.100}$$

wherein

5.4 = total volume (mL) of the coupled reaction

0.003 = the change in A_{253} per minute of trypsin as per the unit definition

0.200 = volume (mL) of the enterokinase/trypsinogen solution added to the N-benzoyl-L-arginine ethyl ester hydrochloride substrate solution

P.A. = Potential Activity of trypsinogen that is a reported value found on the product label of trypsinogen

0.024 = mg of trypsin per nanomole of trypsin

15 = time (min) of the enterokinase/trypsinogen reaction

0.100 = volume (mL) of enterokinase solution used

HAART drug solutions were mixed with the substrate prior to incubation and addition of enzyme. AGENERASE®, NORVIR®, SUSTIVA®, VIRAMUNE® and VIRACEPT® were dissolved in DMSO. All other HAART drugs were dissolved in distilled water.

Results

Table III contains the results for the inhibition of lipase by protease inhibitors and Table IV summarizes the IC_{50} values. AGENERASE® (amprenavir) solution, AGENERASE® capsules, FORTOVASE® (saquinavir), NORVIR® (ritonavir) and VIRACEPT® (nelfinavir mesylate) exhibited >30% inhibition of pancrelipase at physiological concentration. CRIXIVAN® (indinavir sulfate) exhibited no significant inhibition of pancrelipase, while INVIRASE® (saquinavir mesylate) showed non-specific inhibition. Three different INVIRASE® concentrations were tested and resulted in the same percent inhibition of pancrelipase indicative of a non-specific interaction.

TABLE III

INHIBITION OF LIPASE BY HAART DRUGS

Trade Name	Rec. Dose (mg)	Aqueous Solubility (mg/mL)	Physio. Conc.* (μg/mL)	Drug Tested (μg/mL)**	Inhibition of Lipase (%)
PROTEASE INHIBITORS (PI)					
AGENERASE® Solution	1400	0.04	2800	2500 500 100 50 10	99 99 79 33 4
AGENERASE® Capsules	1200	0.04	2400	2500 500 250	100 74 58
NORVIR®	600	Insol.	1200	2670 1300 670 70	71 76 36 15
VIRACEPT®	750	Insol.	500	3330 1670 420 100	100 71 52 21
FORTOVASE®	1200	Insol.	2400	6670 4000 2330 1330 660	96 70 43 32 12
INVIRASE®	600	2.2	1200	1610 1290 161	21 24 24
CRIXIVAN®	800	Sol.	1600	4270 1600	6 0

TABLE III (contd.)

Trade Name	Rec. Dose (mg)	Aqueous Solubility (mg/mL)	Physio. Conc.* (μg/mL)	Drug Tested (μg/mL)**	Inhibition of Lipase (%)
NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTI)					
COMBIVIR®***	150L 300Z	20	 300L 600Z	933L 2000Z 397L 799Z	9 15
EPIVIR®	150	70	 300	1310 263	9 0
HIVID®	0.75	76	1.5	5	0
RETROVIR®	300	20	60	1330	0
VIDEX®	400	27	 800	2670 800	5 1
ZERIT®	40	83	80	80	0
ZIAGEN®	300	77	 600	1320 645 376	7 13 4
NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTI)					
RESCRIPTA®	400	Insol.	 800	1490 896	34 18
SUSTIVA®	600	Insol.	 1200	3330 1330	64 26
VIRAMUNE®	200	Insol.	 400	1330 400	13 3

* Physiological concentration is calculated as follows: recommended dose (mg)/approx. volume in the duodenum (500 mL)

** Mass of drug based upon label claims: AGENERASE® solution was used neat; AGENERASE® capsules, INVIRASE®, NORVIR®, RESCRIPTA® and VIRAMUNE® were dissolved in 50% methanol; SUSTIVA® was dissolved in 70% methanol; VIRACEPT® was dissolved in 100% methanol. All NRTI were dissolved in distilled water.

*** COMBIVIR® is a mixture of the active drugs Lamivudine (L) and Zidovudine (Z).

Based on the results shown in Table III further studies were conducted on various HAART drugs. The methodology and results follow hereunder.

TABLE IV

IC_{50} VALUES FOR INHIBITORS OF PANCRELIPASE AT PH 9.0

Compound Tested	IC_{50} (μg/mL)	IC_{50} (μM)
AGENERASE® solution	89	176
AGENERASE® capsules	368	787
NORVIR®	381	528
VIRACEPT®	443	780
FORTOVASE®	2164	3226
TPGS	275	182
ORLISTAT	0.22	0.44

None of the NNRTI or NRTI showed significant inhibition of pancrelipase at physiological concentrations. At a concentration three times physiological level, SUSTIVA® (efavirenz) exhibited 64% inhibition of pancrelipase.

Table V contains the results of the excipients that were tested for inhibition of pancrelipase activity. The excipients contained in the drug preparations that resulted in >30% pancrelipase inhibition were tested for pancrelipase inhibition. The only excipient that resulted in significant inhibition of pancrelipase activity was TPGS, which is contained in both AGENERASE® solution and AGENERASE® capsules.

TABLE V

INHIBITION OF PANCRELIPASE AT pH 9.0 BY EXCIPIENTS

Excipient	Drugs Containing Excipient	Aqueous Solubility (mg/mL)	Max. Amount in Prep (wt%)	Wt % Excipient in Source Used (100%=pure)	% Inhibition of Pancrelipase
PEG-400	AGENERASE®*	Soluble	30	100	0
	NORVIR®		30	100	0
Povidone	INVIRASE®	Soluble	25	40	17
TPGS	AGENERASE®*	200		20 4 2 1 0.5 0.2 0.02	96 95 93 79 43 6 0
Vitamin E	FORTOVASE®	Insoluble	0.005	50	20

* Both solution and capsules contain PEG-400 and TPGS

The results in Table V indicate that certain HAART drugs and/or their excipients inhibit pancrelipase.

Table VI illustrates the inhibition of pancrelipase by AGENERASE®, FORTOVASE®, NORVIRx®, VIRACEPT®, and TPGS that can be overcome by the addition of excess lipase.

TABLE VI

REACTIVATION OF DRUG INHIBITED PANCRELIPASE BY THE ADDITION OF EXCESS LIPASE

Drug	% Pancrelipase Activity	% Reactivation of Pancrelipase
AGENERASE®	62	105
FORTOVASE®	65	110
NORVIR®	70	110
VIRACEPT®	35	50
TPGS	55	98

Colipase Results

In order to determine the lipase inhibitory mechanism of the protease inhibitors, colipase was added to reaction mixtures that exhibited >30% inhibition of pancrelipase. Table VII illustrates that colipase had no effect on the inhibition of pancrelipase when added to reaction mixtures that contained USP lipase reference standard, NORVIR®, VIRACEPT® and Orlistat. Pancrelipase inhibition was reversed, i.e. pancrelipase reactivated when colipase was added to the reaction mixtures that contained AGENERASE® (capsule and solution dosage) and TPGS. The percent of pancrelipase activity increased from 21% to 79% when excess colipase was added to a reaction mixture that contained TPGS.

TABLE VII

REACTIVATION OF DRUG INHIBITED PANCRELIPASE BY THE ADDITION OF COLIPASE

Drug Tested	Drug Conc. (mg/mL)	% Lipase Activity No Colipase	% Lipase Activity with Colipase
USP Reference PANCRELIPASE	0.4	100	97
AGENERASE® capsules	1.5	27	74
Solution	7.5	20	63
NORVIR®	2.5	14	15
VIRACEPT®	5.4	15	17
FORTOVASE®	2.3	15	26
TPGS	10.0	21	79
ORLISTAT	0.002	37	29

The inhibition of Enterokinase and trypsin by PI, NNRTI and NRTI are summarized in Tables VIII, IX and X.

As illustrated in Tables VIII, IX and X, HAART drugs did not significantly inhibit either enterokinase or trypsin activity indicating there is no interference with the zyomogen activating cascade. Therefore, the conversion of procolipase to colipase may not be effected by the HAART drugs.

TABLE VIII

INHIBITION OF ENTEROKINASE BY PROTEASE INHIBITORS (PI)

Trade Name	Chemical Name	Recommended Dose (mg)	Aqueous Solubility Mg/mL	Phys. Conc.* (μg/mL)	Drug Tested (μg/mL**)	Drug Tested Unit of Enterokinase μg/U	Inhibition of Enterokinase (%)
CRIXIVAN®	Indinavir Sulfate	800	Soluble	1600	157	20.1	0
AGENERASE® Capsule	Amprenavir	1200	0.04	2400	111	16.4	0
INVIRASE®	Saquinavir Mesylate	600	2.22	1200	169	21.7	0
VIRACEPT®	Nelfinavir Mesylate	750	Insoluble	1500	125	17.8	0

* Physiological concentration is calculated as follows: recommended dose (mg/approx. volume in duodenum (500 mL))

** AGENERASE® capsule and VIRACEPT® were dissolved in DMSO, while CRIXIVAN® and INVIRASE® were dissolved in distilled water

Table IX contains the result of inhibition of Trypsin by Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI).

TABLE IX

NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NNRTI)

Trade Name	Chemical Name	Recommended Dose (mg)	Aqueous Solubility Mg/mL	Phys. Conc.* (μg/mL)	Drug Tested (μg/mL**)	Drug Tested Unit of Trypsin μg/U	Inhibition of Trypsin (%)
RESCRIPTA®	Delavirdine mesylate	400	Insoluble	800			
SUSTIVA®	Efavirenz	600	Insoluble	1200	66	649	0
VIRAMUNE®	Nevirapine	200	Insoluble	400	56	710	0

* Physiological concentration is calculated as follows: recommended dose (mg/approx. volume in duodenum (500 mL))

** SUSTIVA® and VIRAMUNE® were dissolved in DMSO

Table X contains the result of inhibition of Trypsin by Nucleoside Reverse Transcriptase Inhibitors (NRTI).

TABLE X

NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTI)

Trade Name	Chemical Name	Recommended Dose (mg)	Aqueous Solubility Mg/mL	Phys. Conc.* (μg/mL)	Drug Tested (μg/mL**)	Drug Tested Unit of Tryspin μg/U	Inhibition of Tryspin (%)
EPIVIR®	Lamivudine	150	70	300.0	48	763	0
HIVID®	Zalcitabine	0.75	76	1.5			0
	Zalcitabine USP Ref. STND				44	765	0
RETROVIR®	Zidovudine	300	20	60.0			0
	Zidovudine USP Ref. STND				56	773	0
ZERIT®	Stavudine	40	83	80.0	47	741	0
ZIAGEN®	Abacavir sulfate	300	77	600	141	654	0

* Physiological concentration is calculated as follows: recommended dose (mg/approx. volume in duodenum (500 mL))

** All NRTI were dissolved in distilled water

Summary of In Vitro Studies

Based on these *in vitro* results, FORTOVASE®, NORVIR® AND VIRACEPT® inhibited pancrelipase at physiological concentrations. AGENERASE® capsule exhibited 99% inhibition of pancrelipase at physiological concentration (2800 μg/mL) and AGENERASE® solution exhibited 100% pancrelipase inhibition at physiological concentration (2400 μg/mL). TPGS also significantly inhibited pancrelipase. FORTOVASE® exhibited approximately 74% inhibition of pancrelipase at physiological concentration (2400 μg/mL). NORVIR® exhibited approximately 73% inhibition of pancrelipase at physiological concentration (1200μg/mL). VIRACEPT® exhibited approximately 72% inhibition of pancrelipase at physiological concentration (1500μg/mL). This inhibition can be overcome by addition of excess pancrelipase. The addition of excess colipase to reaction mixtures, inhibited by AGENERASE® formulations and TPGS, restored pancrelipase activity indicating that the TPGS and/or amprenavir interfered with the lipase/colipase interactions.

The above summarized results indicate the direct inhibition of pancrelipase by protease inhibitors that provides the scientific basis for the administration of a bicarbonate buffered and enteric-coated pancrelipase having a pH of 9.0 to treat HAART-induced diarrhea and steatorrhea.

Treatment of HIV positive patients suffering from HAART induced diarrhea have responded positively (fewer loose stools and reduced incidence of gastrointestinal distention and flatulence) to the administration of an enteric-coated bicarbonate-buffered pancrelipase delayed release capsules. Pancrelipase contains lipases, colipase, amylase, proteases, nucleases and other bioactive substances produced by the pancreatic gland. While the mechanism of action of HAART drug-induced diarrhea is not known, it is hypothesized, based on the above-described results, that the presence of greasy and oily diarrhea is indicative of interference with fat and lipid digestion by HAART drugs and by directly inhibiting pancreatic lipase in the gastrointestinal tract.

The following in vivo studies illustrate the efficacy of PANCRECARB® microspheres when co-administered with HAART drugs in reducing diarrhea in HIV-positive patients.

IN VIVO STUDIES

Objective

The purpose of this study was to determine the safety and efficacy of PANCRECARB® enteric-coated microspheres in reducing diarrhea associated with highly active antiretroviral therapy (HAART) in HIV-positive patients.

The primary efficacy variable was the reduction in the frequency of diarrhea. Frequency of diarrhea is defined as the number of loose and watery stools.

The secondary efficacy measurement was the effect on gastrointestinal symptoms of malabsorption i.e., pain, gas, and bloating as well as overall satisfaction with PANCRECARB® enteric-coated microspheres.

Introduction

As mentioned earlier, a large number of HIV-positive patients (~32%) when treated with antiviral drugs, i.e., Highly Active Antiretroviral Therapy (HAART) have experienced mild to severe diarrhea while on drug therapy. Drug induced diarrhea causes maldigestion with the loss of essential nutrients through the stool, especially fat and fat-soluble vitamins. As a result, patients experience malnutrition, loss of muscle mass and suffer from decreased immuno-competence. Effective correction of diarrhea is critical to the survival and well-being of the patients.

PANCRECARB® (pancrelipase) delayed-release capsules are a digestive supplement produced as bicarbonate buffered and enteric-coated microspheres of lipase, amylase and protease. In theory, PANCRECARB® enteric coated microspheres protect both the bicarbonate and the pancrelipase from inactivation by gastric acid. The enzyme microspheres are designed to allow safe delivery of the bicarbonate and the enzymes to the upper small intestine, where the bicarbonate is released to increase the pH in the microenvironment surrounding the microspheres to

a range of 8.5 to 9, i.e., a range that provides optimal lipase activity for digestion of fats and lipids. Therefore, it is believed that PANCRECARB® with its unique enteric-coated enzyme formula will improve digestion and absorption of fat and will aid the HIV-Positive patient in the control of diarrhea.

The in vivo studies were conducted as follows.

All selected patients were HIV-positive with a CD4 count of >100 cells/μL. The main inclusion criteria included: 1) did not experience diarrhea (≥3 loose and watery stools/day prior to HAART) and experienced diarrhea (≥3 loose and watery stools/day) for ≥4 days while on HAART; and 2) HAART-induced diarrhea successfully managed by pancrelipase therapy.

Protocol No. 092100 was a double-blind, single-site and randomized study to evaluate the efficacy of PANCRECARB® as compared to a placebo in reducing antiviral drug induced diarrhea in HIV-Positive patients. Patients were evaluated during two 7-day treatment phases using a crossover design. A one(1) day washout period was used between the two (2) treatment phases. All patients discontinued the use of any anti-diarrheals that they were taking prior to the screening phase. The placebo was an enteric-coated formulation in microsphere form that is similar in appearance to PANCRECARB®, but without the pancrelipase.

Initially, thirteen (13) patients were enrolled and received the study drug. One patients was discontinued due to non-compliance and a second patients was determined to be a protocol violation due to having too few stools during the screening period. Therefore, eleven (11) patients successfully competed the study and were included in the analysis of efficacy evaluable patients and 13 patients, all of whom received study medication, are included in the intention to treat (ITT) population and the analysis of safety.

Patients were required to visit the clinic four (4) times during the study at the following times:

a) Screening Visit (at least one (1) week prior to study entry)

b) Initiation of Treatment Phase 1 (Day 1)

c) Washout day and prior to Initiation of Treatment Phase 2 (Day 8)

d) End of Study Visit (Day 16)

Throughout the study, patients were required to keep a "Daily Diary Record for Digestive Symptoms" and also on Day 3 and Day 7 of the Treatment Phases, they completed a "Satisfaction Survey".

Statistical Methods: Baseline patient characteristics including demographics and most recent CD4 count were summarized. Treatment efficacy and other diary variables were evaluated using the paired t-test. Comparisons between the treatments were made on data from individual days, over the last 3 days of treatment and over the 7-day study period.

On the last day of treatment, the number of formed stools signifying less diarrhea approached statistically significance favoring PANCRECARB® treatment in comparison with the placebo treatment ($p=0.053$). There was no statistically significant difference between the treatment groups for the number of stools for the last three days of treatment. However, there was a trend for more formed and less watery stools, hence less diarrhea in the PANCRECARB® group. The outcomes from both patient and clinician surveys showed that there was more satisfaction with the PANCRECARB® treatment.

There was no treatment-related adverse events during the study. Additionally, no statistical differences in daily dairy gastrointestinal symptoms were observed between the two arms.

As a follow-up to Protocol No. 092100, patients that successfully completed the study were requested to participate in a telephone survey. Of the eleven (11) patients that qualified for the telephone survey, eight (8) participated.

RESULTS OF THE TELEPHONE FOLLOW-UP SURVEY TO PROTOCOL NO. 092100

As mentioned above, eight (8) patients were contacted by telephone to complete a questionnaire comparing their experiences before PANCRECARB® therapy and then three (3) or more months after being on PANCRECARB® therapy.

The survey and summaries address the following variables: Stool Frequency / 24 Hours, Bowel Movement and its Interference with Work, Urgency of Bowel Movement, Stool Consistency, Gastrointestinal Symptoms of Malabsorption i.e., Pain, Gas, and Bloating, and Quality of Life.

FREQUENT STOOLING:

Patients were asked if they experienced "frequent stooling" prior to PANCRECARB® therapy (i.e. 4 or more stool /day) and after PANCRECARB® therapy if they experienced a reduction in "frequent stooling" (i.e., 3 or less stools/day). Before PANCRECARB®, 6 of 8 patients experienced "frequent stooling" and 7 of 8 experienced a reduction in frequent stooling / 24 hours after PANCRECARB®. One patient experienced no change. Therefore, 87% of patients experienced a reduced number of stools / 24 hours while on PANCRECARB® therapy. This shown in Fig. 1 and Fig. 2 entitled "Stool Frequency / 24 Hours".

INTERFERENCE WITH WORK

Prior to PANCRECARB® therapy, 7 of 8 patients said that their daily bowel movements interfered with their work schedule. Three (3) patients (37.5%) stated that bowel movements "very much interfered", 3 patients (37.5%) said there was "some interference", one (1) patient (12.5%) said he "could not work", and one (1) patient (12.5%) did not experience any interference.

After a minimum of 3 months on PANCRECARB® therapy, 2 of the patients that experienced bowel movements which "very much interfered" with work now experienced only "some interference". The third patient that stated "very much interfered" now said he had "no interference" after PANCRECARB® therapy. Of the 3 patients that said they experienced "some interference" before PANCRECARB®

therapy, 2 now have "no interference" and there was no change with the third patient. The patient that initially had "no interference" remained the same. The final patient that "could not work" experienced the biggest change by having "no interference" with work after PANCRECARB® therapy. This is shown in Fig. 3.

Therefore, 6 of 8 patients (75%) experienced a decrease in the rate of "interference with work" after being on PANCRECARB® therapy for at least 3 months. This is shown in Fig. 4.

URGENCY OF BOWEL MOVEMENT:

Urgency of bowel movement was addressed in two ways. First, as a percentage in the improvement (reduction) that the patients experienced and second as a "reduction in the "Severity" of the "Rate of Urgency".

Of the eight (8) patients questioned regarding "urgency of bowel movement" before PANCRECARB® therapy, one (1) patient experienced "mild" urgency, 5 patients indicated they had "severe " urgency, one (1) patient had "maximum" severity, and one (1) patient had "no" urgency of bowel movement. After 3 months on PANCRECARB®, 6 of 8 patients (75%) saw an improvement (reduction) in the "Urgency of Bowel Movement". With the 2 patients that saw no improvement, one patient remained the same at "no" severity and the second patient changed from "mild" severity to "maximum" severity. This is shown in Fig. 5.

To determine the reduction in "Severity" of the "Rate of Urgency" of bowel movements before PANCRECARB® therapy and after at least 3 months of PANCRECARB® therapy a weighted average of the difference between patients before and after PANCRECARB® therapy was conducted.

Rate of Urgency:	Before PANCRECARB® therapy %	After PANCRECARB® therapy %
None	12.5 (1 of 8 patients)	50.0 (4 of 8 patients)
Mild	12.5 (1 of 8 patients)	37.5 (3 of 8 patients)
Severe	62.5 (5 of 8 patients)	0.0
Max Severity	12.5 (1 of 8 patients)	12.5 (1 of 8 patients)

Patient ID:	1	2	3	4	5	6	7	8		
Before Therapy:	2	4	1	2	2	0	2	2	=	15
After:	1	1	4	0	0	1	0	0	=	7

$$15 - 7/15 \text{ X } 100\% = 53\%$$

Therefore patients experienced a 53% reduction in the "Severity" of the "Rate of Urgency" of bowel movements while on PANCRECARB® therapy. This is shown in Fig. 6 and Fig. 7.

STOOL CONSISTENCY:

Patients were asked if they experienced a change in "stool consistency", i.e. from "loose/watery" to "formed". As mentioned previously, diarrhea, for this study, was defined as the number of loose and watery stools. Therefore, "stool consistency" in this questionnaire can also be defined as a change in diarrhea.

After the patients were on PANCRECARB® therapy for 3 or more months, 7 of 8 patients (87%) said they experienced a change in "stool consistency", i.e. less diarrhea. This is consistent with the study protocol in which there was a trend toward more formed and less watery and loose stools and hence less diarrhea in the PANCRECARB® group. This is illustrated in Fig. 8.

GASTROINTESTINAL SYMPTOMS:

During the course of the study 4 of 8 patients (50%) indicated they experienced gastrointestinal symptoms, i.e. abdominal cramps and pain, with digestion of a meal before PANCRECARB® therapy. After PANCRECARB® therapy, the same 4 patients indicated that they continued to experience gastrointestinal symptoms.

QUALITY OF LIFE:

Consistent with the results from the Protocol 092100 study, 7 of 8 patients (87%) said they experienced an improvement in the "Quality of Life" while being on PANCRECARB® therapy. One (1) patient (13%) indicated he did not experience a change, two (2) patients (25%) said their life was very much improved, and five (5) patients (62%) indicated their life somewhat improved. Therefore, assessment of patient satisfaction showed that PANCRECARB® treatment had more patients with a better outcome as compared to the placebo treatment. This is illustrated in Fig. 9.

CONCLUSION:

The results of this study demonstrated that 87% of the HIV patients with HAART induced diarrhea experienced an improvement in the "Quality of Life" while being treated with PANCRECARB® capsules. Additionally, 87% of the patients experienced a reduced number of stools / 24 hours and had less diarrhea, 75% of patients experienced a decrease in the rate of "interference of bowel movement" while at work, and 53% of the patients experienced a reduction in the "severity" of the "Rate of Urgency" of bowel movements while on PANCRECARB® therapy.

Based on the results of this study, it is concluded that PANCRECARB® therapy is effective in reducing the severity of HAART induced diarrhea in HIV afflicted patients.

As stated in the Summary of the Invention, a gastric acid-resistant polymer-coated, buffered digestive enzyme/ursodeoxycholate composition containing from about 10% to about 90% of a concentrate of an enzyme selected from the group consisting of pancreatic proteases, pancreatic lipases, colipase, pancreatic nucleases, pancreatic amylases and other bio-active substances produced by the pancreas will reduce/eliminate diarrhea in HIV positive patients being treated with HAART drugs when co-administered or sequentially administered with said drugs.

The physicians treating HIV-positive patients under the HAART protocol will determine the frequency and amount of lipase-containing compositions necessary to

counteract the diarrhea occurrence. This determination by the physician will depend on the extent of diarrhea, the cocktail of HAART drugs, and the general health of the patient. Compositions other than that disclosed in U.S. Patent No. 5,578,304 include compositions disclosed in the following patents (all of which are incorporated herein by reference):

U.S. Patent No. 5,460,812 discloses compositions in which there is about 10 to about 90.0% w/w of a concentrate of an enzyme selected from the group consisting of pancreatin, pancreatin proteases, pancreatic lipases, pancreatic nucleases, pancreatic amylases and other bio-active substances produced by the pancreas. The compositions include about 0.3 to about 75% of a bile salt and a buffering agent.

U.S. Patent No. 5,324,514 discloses a composition comprising of from about 71 to 90% w/w of a concentrate of an enzyme selected from the group consisting of pancreatic proteases, lipases, nucleases, and amylases; of from about 0.3% to about 13% w/w of a bile salt; and of from about 0.8% to about 5% w/w of a buffering agent.

U.S. Patent No. 5,260,074 discloses a digestive enzyme and bile salt composition comprising:

of from about 71 to about 90% w/w of an enzyme selected from the group consisting of pancreatic proteases, lipases, nucleases and amylases;

about 1.0 to about 61% w/w of a salt of ursodeoxycholic acid selected from the group consisting of sodium, potassium, ammonium, tromethamine, ethanolamine, diethanolamine, and triethanolamine;

about 0.8 to about 5.0% w/w of a buffering agent;

about 0.3 to about 19% w/w of an adhesive polymer selected from the group consisting of hydroxypropyl cellulose, polyvinylpyrrolidone, cellulose acetate, phthalate and methyl cellulose;

about 0.9 to about 16% w/w of a disintegrant selected from the group consisting of starch, modified starches, microcrystalline cellulose and propylene glycol alginate; and

a gastric acid-resistant polymer coating the listed ingredients which disintegrates under neutral or basic conditions.

U.S. Patent Nos. 5,578,304, 5,460,812, 5,324,514 and 5,260,074 relate to gastric acid-resistant compositions in which the coatings on the compositions do not release the active ingredients in the acidic pH of the stomach, but dissolve in the neutral or slightly basic environment of the intestines in which the active ingredients are then released.

The compositions and methods disclosed in the aforementioned patents are directed to the treatment of digestive disorders, pancreatic enzyme insufficiency, impaired liver function, cystic fibrosis, regulating the absorption of dietary iron and cholesterol and for dissolving gallstones. None of these patents suggest the use of these compositions to reduce and/or eliminate diarrhea/steatorrhea in HIV-positive patients treated with HAART. The compositions do, however, contain lipase and co-lipase and other bio-active substances produced by the pancreas which, as shown above, helps to reduce and/or eliminate diarrhea/steatorrhea in HIV-positive patients.

Other compositions containing pancreatin coated with gastric juice-resistant polymers may also be used (see for example, U.S. Patent Nos. 4,280,971 and 5,378,462, which are incorporated herein by reference).

Various modifications of the present invention disclosed will become apparent. This invention is intended to include such modifications to be limited only by the scope of the claims.